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RETINAL VESSEL PHENOTYPE IN PATIENTS WITH PRIMARY OPEN-ANGLE GLAUCOMA

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Keywords:	glaucoma, retinal vessel, tortuosity, fractal dimension, vampire software



**SERVICE
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Grenoble, 10/12/2018

Dear Editor,

Pr C. Chiquet
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Please find attached a full-length paper entitled:
**" RETINAL VESSEL PHENOTYPE IN PATIENTS WITH PRIMARY
OPEN-ANGLE GLAUCOMA"** which we wish to submit to the
Journal Acta Ophthalmologica.

Pr F. Aptel
Dr D. Bernheim
Dr K. Palombi
Praticiens Hospitaliers
Dr R. Semecas
Dr J. Kosacki
Dr M. Gallice
Chefs de Clinique - Assistan

All authors have seen and approved the manuscript and have
significantly contributed to this work. The manuscript has not been
published and is not being considered for publication elsewhere.

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This original case-control study provides new perspectives on the
relationship between glaucoma and phenotype of retinal vessels.
This study showed that the morphology of the retinal vessels
differed in POAG patients compared to healthy subjects. POAG was
associated with a narrowing of arterial and venous retinal vessels, a
higher arterio-venule ratio, and lower values of fractal parameters
(marker of the anatomical complexity of the vessel network). Central
retinal artery equivalent was positively associated with RNFL
thickness but not visual field parameters.

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The important insights of this study would promote longitudinal study
or interventional study given the opportunity to test whether
changing retinal vasculature could have an impact on the
progression of glaucoma.

Sincerely yours,

Hôpital MICHALLON
8ème étage

Prof. Christophe CHIQUET, MD, PhD

RETINAL VESSEL PHENOTYPE IN PATIENTS WITH PRIMARY OPEN-ANGLE GLAUCOMA

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Running head: RETINAL VESSELS IN PRIMARY OPEN-ANGLE GLAUCOMA

Abstract

Purpose: To characterize the phenotype of retinal vessels using central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), tortuosity and fractal dimension (FD) in primary open angle glaucoma (POAG) subjects.

Methods: This prospective case control multicenter study included 61 POAG subjects and 61 controls matched for age, systemic hypertension and body mass index. Fundus images of the right-eye were acquired using a non-mydriatic camera. CRAE, CRVE, arteriole-to-venule ratio, FD and tortuosity of the vascular network were measured using VAMPIRE software (Vessel Assessment and Measurement Platform for Images of the Retina). POAG patients underwent 24.2 sita-standard visual field and peri-papillary Optical Coherence Tomography examinations. Data were expressed as median and interquartile range (75-25th percentiles).

Results: The control group was comparable to the POAG group for sex ratio, refraction and intraocular pressure. The mean CRAE and the mean CRVE were significantly lower in the POAG group than in the control group (150.5 (137.9; 157.1) μm vs 161.3 (154.0; 168.4) μm and 204.8 (190.1; 218.1) μm vs 233.5 (222.3; 246.9) μm , respectively; $p < 0.001$) and for fractal parameters as well. No significant difference was found for tortuosity between the two groups. There was a significant correlation between CRAE and retinal nerve fiber layer thickness ($r = 0.27$; $p = 0.03$). VAMPIRE parameters were not correlated with visual field indices.

Conclusion: POAG was associated with a narrowing of arterial and venous retinal vessels, a higher arterio-venule-ratio, and lower values of fractal dimension. The relationship between CRAE and RNFL thickness needs further investigation.

Introduction

Primary open angle glaucoma (POAG), characterized by a progressive change of the optic nerve head and consequent visual field defects, is the second cause of blindness worldwide. Several risk factors have been associated with glaucoma, including increased intraocular pressure (IOP), which is the only treatable risk factor to date, ethnicity, myopia, and age. The implication of vascular factors is still debated. Optic nerve vascularization shares with retinal vascularization an effective autoregulation, and the absence of sympathetic innervation. Reduction of ocular perfusion pressure, associated with deteriorated vascular autoregulation, could promote ischemia and oxidative stress within the optic nerve.

Clinical studies of ocular vascular changes associated with glaucoma have mainly focused on retinal vessel diameter (Jonas et al. 1989; Ikram et al. 2005; Mitchell et al. 2005; Amerasinghe et al. 2008; Kawasaki et al. 2013; Yoo et al. 2015), and the relationship with retinal nerve fiber layer (RNFL) thickness or visual field defects (Hall et al. 2001; Zheng et al. 2009; Tham et al. 2013). For instance, a positive association was reported between decreased peripapillary arteriole diameter and visual field defects in the corresponding hemifield (Hall et al. 2001). Moreover, a narrower retinal arteriolar and venular caliber was reported in POAG patients independently of IOP levels (Mitchell et al. 2005; Amerasinghe et al. 2008). A recent longitudinal study also reported that retinal arteriolar narrowing was associated with an increased 10-year risk of glaucoma development (Kawasaki et al. 2013). The study based on retinal vascular diameters can be improved by analyzing fractal measurements that provide information about the complexity of the vessel network (McGrory et al. 2017). More recently OCT angiography was used to image the microvasculature in the superficial and deep capillary layers in the peripapillary

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region. Glaucoma patients had a lower vessel density than controls (Yarmohammadi et al. 2016), and vessel density was correlated with visual field loss or RNFL thickness (Lee et al. 2016).

Analysis of fundus camera images offers a non-invasive measurement method to study the vascular retinal network, which reflects the efficiency of the circulation and the distribution of shear stress. VAMPIRE (Vessel Assessment and Measurement Platform for Images of the Retina, Universities of Edinburgh and Dundee) software enables quantitative analysis of the vascular morphometry. It has been used in several studies including lacunar stroke, among others (Perez-Rovira et al. 2011; MacGillivray et al. 2015; Trucco et al. 2015; McGrory et al. 2017).

The objective of this study was to characterize the phenotype of retinal vessels using recognized retinal vascular parameters - central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), tortuosity and fractal dimension (FD) - in eyes with POAG compared to controls matched for age, systemic hypertension and body mass index (BMI).

Material and methods

Participants

Patients were prospectively included in two separate cohorts at the University Hospital of Dijon and at the University Hospital of Grenoble (IRB #5891). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki on medical research in patients. Subjects were included after they provided written and oral informed consent.

Primary open-angle glaucoma population

The group of patients suffering from POAG consisted in 61 subjects over 18 years old (median: 61; 25-75 percentiles: (55.5; 71.5) years), with a sex-ratio women / men of 1.1, a median BMI of 22.6 (20.5; 25.1), systemic hypertension in 19.7%, a median refraction of -0.5 (-1; +1) diopters and a mean IOP of 14 (11.5; 17) mmHg (under treatment). Previous treatment history of POAG patients is summarized in [Table 2](#).

Fifty-one patients (83%) were treated with at least one hypotensive topical medication. For visual field, median MD and PSD were -4.4 (-13; -1.4) dB and 5.8 (2.6; 9.1) dB, respectively. RNFL average was 69 (58.5; 77.0) μm .

Diagnosis of glaucoma was based on consecutive and reliable abnormal standard automated perimetry with abnormal results on the Glaucoma Hemifield Test and pattern standard deviation outside 95% of normal limits, and characteristic optic nerve damage (asymmetric cup-to-disc ratio >0.2 , rim thinning, notching, excavation or retinal nerve fiber layer defect). A gonioscopy ruled out patients with non-open angles and we excluded patients with secondary glaucomas or normal tension glaucomas. Humphrey 24.2 sita-standard visual field parameters (Mean Deviation and Pattern Standard Deviation) were recorded and the reliability indices of the

European Glaucoma Society were used: visual fields with fixation loss lower than 20%, false-positive errors lower than 33%, and false-negative errors lower than 33% were considered as reliable. Optical Coherence Tomography examinations parameters (peripapillary and sectorial retinal nerve fiber layer thickness (Cirrus HD-OCT 5000, Zeiss Meditec, Dublin, CA, USA) were performed. Glaucoma IOP-lowering treatments were also reported.

Control group

Sixty-one controls were included and were matched to the POAG patients for age (5 year-interval), systemic hypertension, and BMI. These controls originated from the 2 clinics where glaucoma patients came from. Exclusion criteria were: pregnant or lactating women, aged less than 18 years, major person under guardianship or unable to consent, patients with an ametropia > 3 diopters (spherical equivalent), or with any ocular disease. Medical history was collected on the basis of self-declaration, particularly cardiovascular risk factors high blood pressure, diabetes, dyslipidemia and smoking. Visual acuity (VA, LogMar), axial length (IOL master, Zeiss Meditec™, Dublin, CA, USA), IOP (non-contact tonometry; TONOREF II, Nidek™, Gamagori, Japan), anterior segment slit lamp examination and fundus examination were performed to rule out ocular diseases. The control group was comparable to the POAG group for age (66 (56; 71) years, $p=0.9$), sex ratio (1.44; $p=0.4$), BMI (23.8 (21.7; 23.8), $p=0.8$), systemic hypertension (21.3%, $p=0.8$), refraction (0 (-0.8; 0.75) diopters, $p=0.2$) and IOP (15 (13; 16) mmHg, $p=0.4$).

Acquisitions and analysis using 30 or 45-degree fundusoscopic color photograph

30 or 45-degree fundus camera images of the right-eye were acquired, centered on the optic nerve and the macula, using a non-mydratic camera: Visucam 200 (Carl Zeiss Meditec™ France) or CR2 (Canon™ Europa, Amstelveen, The Netherlands). Ten images with low quality were excluded in the POAG group due to poor image quality resulting from the presence of cataract or bad fixation.

Image analysis

VAMPIRE measures semi-automatically morphological parameters of the retinal vessels (Perez-Rovira et al. 2011; Trucco et al. 2015). First, the optic disc contour and the macula center were located. This enabled the definition of the usual retinal coordinates (x axis through optic disc (OD) and macula centers, origin in the OD center) and circular zones around the OD, zone A (between OD center and 0.5 optic disc diameter (ODD)), zone B (between 0.5 and 1 ODD), and zone C (between 0.5 and 2 ODD, Figure 1). Manual correction could be performed when the optic disc or fovea have been incorrectly identified automatically. Vessels were subsequently detected and labeled as arterioles or venules semi-automatically (Figure 1).

Here we used CRAE, mean of the widths of the six largest arteries (from the revised formulas of Knudtson)(Knudtson et al. 2003); CRVE, a similarly for the six largest venules (Knudtson et al. 2003); arterio-venule ratio ($AVR = CRAE/CRVE$); FD of the vascular network (a measure of geometric complexity of the pattern of the larger vessels in zone C)(Macgillivray et al. 2007; Doubal et al. 2010), and vascular tortuosity (the average of the six largest arteries and the average of the six largest venules)(Lisowska et al. 2014). AVR, CRAE and CRVE were computed in zone B, fractal measure and vascular tortuosity analysis in zone C. Raw measurements of

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CRAE and CRVE were in pixels.

The pixel-to-mm conversion was as follows: the conversion factor was obtained by dividing the average vertical ODD (over all images, acquired with the same camera at the same resolution) by the assumed average of the disc diameter in microns (1850 μm), as previously described (Varma et al. 1994; Hubbard et al. 1999; Knudtson et al. 2003). The conversion factors for the CANON CR 2 and VISUCAM cameras were 5.77 and 5.82 microns per pixel, respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences program (SPSS 17.0 for Windows, Chicago, IL, USA). In order to study intra- and inter-operator repeatability, 100 healthy and POAG patient’s fundus images were analyzed two times by two operators (RS and OG). The 2-way random average intraclass correlation coefficient (ICC) was used. Quantitative data were expressed as mean with 95% confidence interval of the difference.

VAMPIRE and epidemiological data were expressed as median and interquartile range (75-25th percentiles). Comparisons used the Wilcoxon test (for matched data) and Mann-Whitney test (for independent data). Correlations used the Spearman test. Intra-class correlation coefficients with a confident interval of 95% were calculated, and Bland-Altman plots generated to study repeatability. The tests were two-tailed and statistical significance was set at $p<0.05$. Inter- and intra-operator repeatability for all parameters was excellent, as shown in Table 1.

Results

The mean CRAE and the mean CRVE were significantly lower in the POAG group than in the control group ($p < 0.001$, Table 3). AVR was significantly higher in the POAG group ($p = 0.01$). No significant difference was found for arterial and venous tortuosity between the two groups. POAG patients exhibited reduced values of fractal parameters ($p < 0.001$ for all parameters).

In POAG patients, there was a significant to moderate correlation between CRAE and CRVE ($r = 0.589$; $p < 0.0001$). There was a low to significant correlation between CRAE and RNFL thickness ($r = 0.270$; $p = 0.03$; Figure 2). AVR, CRVE and fractal parameters were not significantly correlated with RNFL. For tortuosity, Amax (the maximum tortuosity measured in an image from the sample of 6 vessels) was significantly correlated with RNFL thickness, but with a low correlation coefficient ($r = 0.353$; $p < 0.005$). CRAE, CRVE, AVR, tortuosity and fractal parameters were not significantly correlated with visual field indices.

Discussion

This case-control study showed that the morphometry of the retinal vessels differed in POAG patients compared to healthy subjects. POAG was associated with a narrowing of arterial and venous retinal vessels, a higher AVR, and lower values of fractal parameters. CRAE was positively associated with RNFL thickness but not visual field parameters. The narrowing of retinal vessels in glaucoma was previously reported in the past, in the peripapillary region (Jonas et al. 1989; Rader et al. 1994; Wang et al. 2007) or at a distance of 2 mm to the optic disc border using a bespoke strategy for measurements of retinal vascular diameters (Wang et al. 2007). More recently, using semi-automatic software and standardized measurements (CRAE,

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CRVE), five independent studies (including between 40 and 127 POAG patients) found POAG associated with reduced retinal vascular diameters (Mitchell et al. 2005; Amerasinghe et al. 2008; Kawasaki et al. 2013; Wu et al. 2013; Zhang et al. 2017). In order to increase the statistical power of comparisons, we carefully compared these glaucoma patients with controls matched for age, BMI and systemic hypertension, well-known factors affecting the retinal vascular calibers. The narrowing of both arterial and venous diameters was observed in our cohort and in these previous studies. In many studies a relationship was shown between glaucoma occurrence and retinal vessels diameters change independently of IOP (Amerasinghe et al. 2008; Kawasaki et al. 2013). This point has not been studied in our population since most of glaucoma patients were already medically or surgically treated.

The AVR allows quantifying the change in vascular diameter taking into account arteries (diameters defined here by CRAE) and veins (CRVE). The higher AVR reported in our study suggests that venular narrowing was predominant. To our best knowledge, this finding is observed for the first time in glaucoma; comparable studies did not report AVR values (Amerasinghe et al. 2008; Kawasaki et al. 2013; Wu et al. 2013; Zhang et al. 2017). Two previous studies reported no association between diagnosed or incident glaucoma and AVR (Ikram et al. 2005; Mitchell et al. 2005). Furthermore, one previous study showed that the retinal vein diameters was not modified in glaucoma patients (Wang et al. 2007). The differential effect glaucoma on veins as compared to arteries should be further studied taken into account other confounding factors such as age and blood pressure values.

Ideally the association between glaucoma and retinal vascular narrowing should be studied longitudinally. At this time, only two studies addressed this point with contrasting results. In the Blue Mountains Eye Study (82 incident glaucoma

patients among a population of 2561 subjects, SIVA software)(Kawasaki et al. 2013), retinal arteriolar (and not venular) narrowing was associated with long-term risk of POAG development (OR = 1.77 ; CI = 1.14 – 3.05). In contrast, the Rotterdam study (74 incident glaucoma patients among a population of 3469 subjects, Retinal Analysis software) showed no relationship between retinal vessel diameters and incident glaucoma with a mean follow-up time of 6.5 years (Ikram et al. 2005). This discrepancy could be related to different populations, definition of glaucoma, semi-automatic softwares and/or follow-up times. VAMPIRE, similar to the SIVA software application, allows the quantification of tortuosity and FD. We did not find that arterial tortuosity was associated with POAG group (Amax). This contrasts with the relationship found in the Singapore Malay Eye Study (SMES) between decreased arteriolar tortuosity and glaucoma (OR 1.73, 95% confidence interval 1.38-2.18, n=123 glaucoma subjects)(Wu et al. 2013). Since only two studies, including ours, quantified tortuosity in different populations (Asian vs Caucasian, severity of glaucoma), more work is needed to assess to clarify this association. The FD captures the complexity of the retinal vascular tree as a geometric pattern, including the degree of branching complexity (Masters 2004). A lower FD value, found in this study and the SMES (Wu et al. 2013), has also been reported in advanced diabetic retinopathy (Grauslund et al. 2010). We notice that fractal measurements have been reported to be associated with age, blood pressure, refractive error and lens opacity (Cheung et al. 2012), and more recently with ganglion cell-inner plexiform layer thickness (Tham et al. 2013). Finally, data strongly suggest that glaucoma is associated with decreased branching complexity. Therefore, the retinal vascular abnormalities are not restricted to the reduced calibers of vessels in the peripapillary region but also are extensive on zone B and C.

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When considering the relationship between retinal macro-vascular parameters and anatomical markers of glaucoma, such as RNFL thickness, an early study by Jonas et al. suggested that retinal vascular narrowing was associated with the severity of glaucoma (Jonas et al. 1989). This is consistent with a previous study in 107 glaucoma patients, showing that retinal venular caliber was independently associated with the temporal- inferior RNFL thickness (Zheng et al. 2009). The relationship between vessel density and RNFL thickness has been also recently described using OCT angiography (Lee et al. 2016). Our study showed also that the CRAE and CRVE were not correlated with the global visual field deficit (MD). One recent study showed that retinal arteriolar diameter is not significantly different in POAG with initial parafoveal scotoma than in those with initial peripheral nasal step (Yoo et al. 2017). One other study showed that the association between the retinal arteries diameters (measured using the retinal vessel analyzer) and MD was generally weak (Resch et al. 2011). A substantial body of literature discusses the discrepancy between structure and function, considering vasculature assessment as a surrogate for structure.

We do acknowledge several limitations to our study. First, the size of our cohort of glaucoma patients is modest. Second, the case-control and the cross-sectional design, even though largely used in the literature, are far from optimal. Third, CRAE and CRVE cannot identify differences in diameters between peripapillary and distal vessels (Rader et al. 1994), between temporal, nasal, inferior or superior segments (Wang et al. 2007). Fourth, the potential variability in magnification error due to different camera has to be taken into account; however it was limited by the use of a pixel-micron conversion factor adjusted to the average optic disc size and the fundus camera.

In conclusion, this case-control study in Caucasians showed that POAG patients exhibited more frequently arteriolar and venular narrowing and a lower FD value. Retinal vessel narrowing was significantly associated with the mean RNFL thickness. Further studies are needed to confirm the longitudinal relationship between optic nerve tissue loss and the retinal vascular phenotype.

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For Peer Review

LEGENDS OF FIGURES

Figure 1. Example of retinal coordinates, zones and vasculature detection and labeling.

Figure 2: Correlation between CRAE (microns) and average RNFL thickness

(microns), $r = 0.27$; $p = 0.03$. CRAE: central retinal artery equivalent; RNFL: retinal nerve fiber layer

For Peer Review

Table 1: Inter- and intra- operator repeatability for two operators, for AVR, CRAE, CRVE and tortuosity (100 images).

CRAE: central retinal artery equivalent; CRVE: central retinal vein equivalent; AVR: arteriole-to-venule ratio; ICC: intraclass correlation coefficient; CI: confidence interval; 1: first operator, 2: second operator.

	ICC 1	CI 95%	ICC 2	CI 95%	ICC inter-operator	CI 95%
AVR	0.923	0.884-0.95	0.823	0.730-0.883	0.918	0.875-0.946
CRAE	0.941	0.910-0.961	0.914	0.869-0.943	0.944	0.915-0.963
CRVE	0.952	0.927- 0.968	0.968	0.952-0.979	0.979	0.968-0.986
Arterial tortuosity	0.969	0.952-0.979	0.958	0.937-0.972	0.972	0.957-0.981
Venule tortuosity	0.98	0.970-0.987	0.962	0.943-0.975	0.992	0.988-0.995

	Previous filtering surgery	Previous SLT	Mono therapy	Bi therapy	Tri therapy	Quadri therapy
Patients (n, %)	17/61 (27.9%)	7/61 (11.5%)	15/61 (24.5%)	22/61 (36%)	13/61 (21.3%)	1/61 (1.6%)

Table 2: Treatment history of the POAG group (n = 61).

SLT: selective Laser trabeculoplasty. Glaucoma hypotensive medications included prostaglandins, beta blockers, carbonic anhydrase inhibitors and/or alpha 2 adrenergic agonists.

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	Controls	Glaucoma subjects
CRAE (microns)	161.3 (154.0; 168.4)	150.5 (137.9; 157.1) *
CRVE (microns)	233.5 (222.3; 246.9)	204.8 (190.1; 218.1) *
AVR	0.69 (0.65; 0.72)	0.73 (0.66; 0.79) **
Arterial tortuosity		
A	0,000034 (0,000013; 0,000126)	0.000049 (0.000021; 0.00014)
Amin	0,000006 (0,000002; 0,000019)	0.0000077 (0.0000024; 0.000033)
Amax	0,000224 (0,000077; 0,000551)	0.00026 (0.00012; 0.00044)
Veins tortuosity		
V	0,000042 (0,000023; 0,000063)	0.000041 (0.000023; 0.00066)
Vmin	0,000006 (0,000002; 0,000014)	0.0000073 (0.0000036; 0.000014)
Vmax	0,000148 (0,000098; 0,000290)	0.00014 (0.00081; 0.00027)
Fractal parameters		
D0a	1.59 (1.54; 1.64)	1.46 (1.39; 1.51) *
D1a	1.58 (1.53; 1.63)	1.46 (1.38; 1.51) *
D2a	1.57 (1.52; 1.62)	1.45 (1.37; 1.50) *
D0v	1.54 (1.50; 1.59)	1.44 (1.39; 1.49) *
D1v	1.53 (1.50; 1.58)	1.42 (1.39; 1.49) *
D2v	1.52 (1.49; 1.57)	1.42 (1.38; 1.48) *
D0tot	1.75 (1.69; 1.77)	1.62 (1.58; 1.69) *
D1tot	1.74 (1.68; 1.77)	1.61 (1.57; 1.61) *

D2tot	1.74 (1.67; 1.76)	1.60 (1.56; 1.67) *
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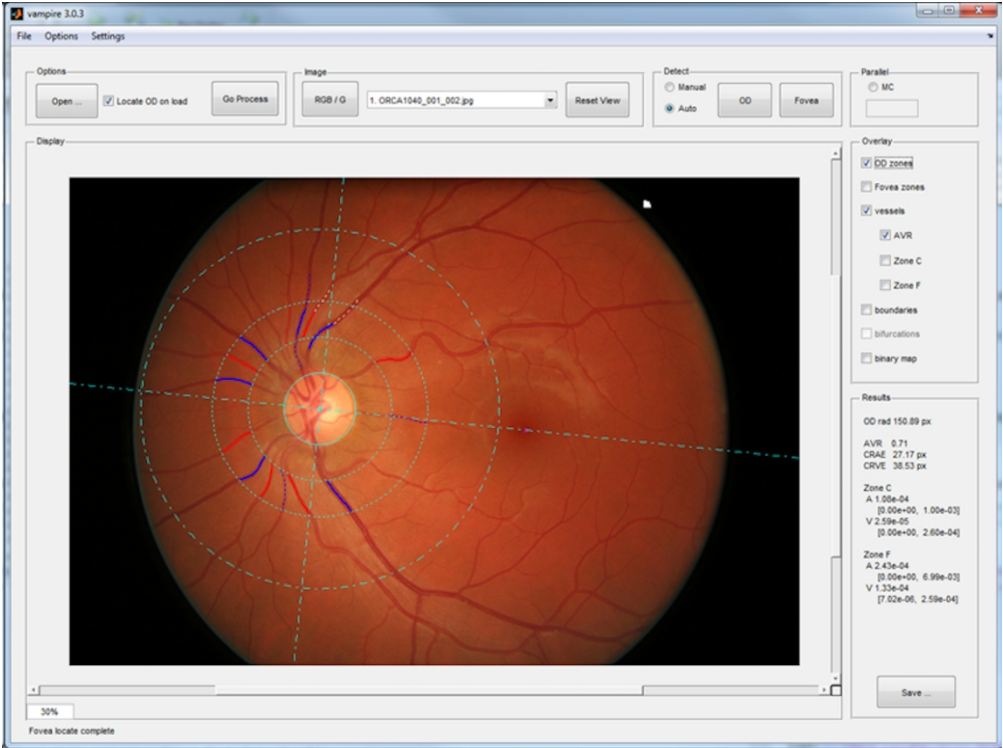
Table 3: Central retinal artery and vein equivalents, the arteriole-to-venule ratio, vascular tortuosity and fractal parameters for the control and glaucoma groups.

Data are expressed as median and interquartile range (75-25th percentiles).

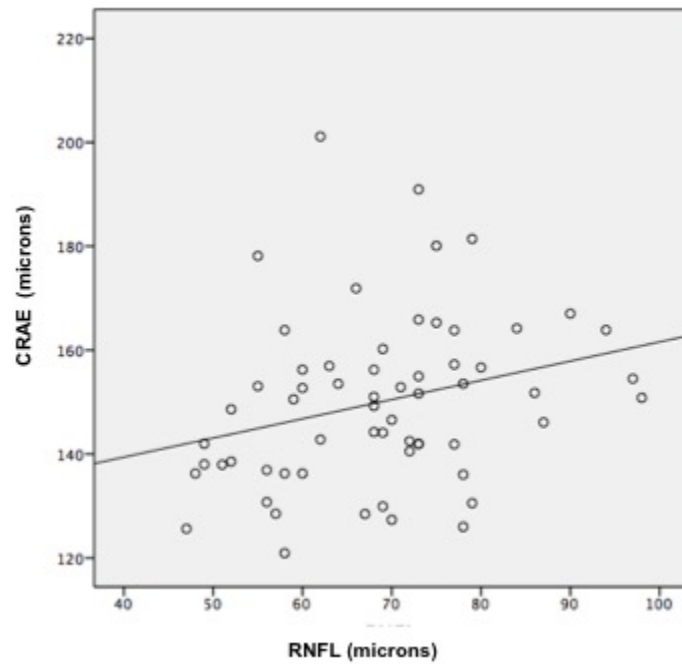
CRAE: central retinal artery equivalent; CRVE: central retinal vein equivalent,

* $p < 0.001$ for the difference between the control and glaucoma group. ** $p = 0.01$

No significant difference was found for arterial and venous tortuosity between the two groups.



Example of retinal coordinates, zones and vasculature detection and labeling.



Correlation between CRAE (microns) and average RNFL thickness (microns)

122x120mm (72 x 72 DPI)